

Appl. No. : 09/965,126
Filed : September 26, 2001

AMENDMENTS TO THE CLAIMS

1. (Currently amended) A method of measuring a holoceruloplasmin concentration in a blood spot comprising:

- a) binding the holoceruloplasmin eluted from a blood spot to a holoceruloplasmin-specific polyclonal antibody and a holoceruloplasmin-specific monoclonal antibody; and
- b) measuring a holoceruloplasmin concentration in the blood spot based on an absorbance standard curve obtained through an assay using a holoceruloplasmin-specific polyclonal antibody and a holoceruloplasmin-specific monoclonal antibody.

2. (Cancelled)

3. (Previously amended) The method of claim 1, wherein the blood spot is collected using a blood filter paper.

4. (Previously amended) The method of claim 1, wherein the ceruloplasmin-specific polyclonal antibody is manufactured from the serum that is obtained by a rabbit immunized with purified human ceruloplasmin containing the holoceruloplasmin.

5. (Previously amended) The method of claim 1, wherein the ceruloplasmin-specific monoclonal antibody is manufactured from a hybridoma cell line obtained through fusion of mouse spleen cells with myeloma cells, selection and cultivation of the fused spleen cells to produce a monoclonal antibody, and wherein the spleen cells were obtained by immunization of a mouse with purified ceruloplasmin containing holoceruloplasmin.

6-9 (Cancelled)

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10. (Previously amended) A method of measuring a holoceruloplasmin concentration in a blood spot according to an enzyme-linked immunosorbent assay, the method comprising the steps of:

manufacturing a ceruloplasmin-specific polyclonal antibody;

manufacturing a ceruloplasmin-specific monoclonal antibody;

conjugating horseradish peroxidase on the ceruloplasmin-specific polyclonal antibody and the ceruloplasmin-specific monoclonal antibody;

manufacturing a standard blood spot and a control reference blood spot by removing a ceruloplasmin from a blood sample and adding a purified ceruloplasmin solution containing a holoceruloplasmin at a constant concentration to the blood sample;

drawing out an absorbance standard curve based on the standard blood spot and the control reference blood spot using the ceruloplasmin-specific polyclonal antibody and the ceruloplasmin-specific monoclonal antibody; and

measuring a holoceruloplasmin concentration in a blood spot of a patient using the standard curve through an enzyme-linked immunosorbent assay.

11. (Previously amended) A method of measuring a holoceruloplasmin concentration in a blood spot according to a dissociation-enhanced time-resolved fluoroimmunoassay, the method comprising the steps of:

manufacturing a ceruloplasmin-specific polyclonal antibody;

manufacturing a ceruloplasmin-specific monoclonal antibody;

conjugating with europium on the ceruloplasmin-specific polyclonal antibody and the ceruloplasmin-specific monoclonal antibody;

manufacturing a standard blood spot and a control reference blood spot by removing a ceruloplasmin from a blood sample and adding a purified ceruloplasmin solution containing a holoceruloplasmin at a constant concentration to the blood sample;

drawing out an fluorescence standard curve based on the standard blood spot and the control reference blood spot using the ceruloplasmin-specific polyclonal antibody and the ceruloplasmin-specific monoclonal antibody; and

measuring a holoceruloplasmin concentration in a blood spot of a patient using the standard curve through a dissociation-enhanced time-resolved fluoroimmunoassay.

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12. (Previously amended) The method of claim 10, wherein the patient has Wilson's disease.

13. (Previously amended) The method of claim 10, wherein the step of removing the ceruloplasmin in the blood is made using a phosphate-buffered saline.

14. (Previously amended) The method of claim 10, wherein the standard blood spot and the control reference blood spot are manufactured by adding a ceruloplasmin with a known concentration to the blood without the ceruloplasmin.

15. (Previously amended) The method of claim 14, wherein the known concentration of the ceruloplasmin has at least 3 different values.

16. (Previously amended) The method of claim 10, comprising a further step of screening an antibody for neutralizing an oxidase activity of the holoceruloplasmin at the step of manufacturing the ceruloplasmin-specific monoclonal antibody.

17. (Previously amended) The method of claim 10, comprising a further step of purifying the antibody after the step of manufacturing the ceruloplasmin-specific monoclonal antibody.

18. (Original) A Wilson's disease screening kit reagent, comprising a holoceruloplasmin-specific polyclonal antibody, a holoceruloplasmin-specific monoclonal antibody, a standard blood spot, and a control reference blood spot.

19. (Currently amended) A Wilson's disease screening kit, ~~being characterized of~~ for measuring a holoceruloplasmin concentration in a blood spot based on an absorbance standard curve obtained through an enzyme-linked immunosorbent assay using a holoceruloplasmin-specific polyclonal antibody, a holoceruloplasmin-specific monoclonal antibody, a standard blood spot, and a control reference blood spot.

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20. (Currently amended) A Wilson's disease screening kit, ~~being characterized of~~ for measuring a holoceruloplasmin concentration in a blood spot based on a ~~an~~ fluorescence standard curve obtained through a dissociation-enhanced time-resolved fluoroimmunoassay using a holoceruloplasmin-specific polyclonal antibody, a holoceruloplasmin-specific monoclonal antibody, a standard blood spot, and a control reference blood spot.

21. (Previously amended) A method of diagnosing Wilson's disease using the screening kit of claim 19.

22. (Previously added) The method of claim 1, wherein the assay is an enzyme-linked immunosorbent assay and the concentration of holoceruloplasmin is based upon an absorbance standard curve.

23. (Previously added) The method of claim 1, wherein the assay is dissociation-enhanced time-resolved fluoroimmunoassay and the concentration of holoceruloplasmin is based upon a fluorescence standard curve.

24. (Previously added) The method of claim 11, wherein the patient has Wilson's disease.

25. (Previously added) The method of claim 11, wherein the step of removing the ceruloplasmin in the blood is made using a phosphate-buffered saline.

26. (Previously added) The method of claim 11, wherein the standard blood spot and the control reference blood spot are manufactured by adding a ceruloplasmin with a known concentration to the blood without the ceruloplasmin.

27. (Previously added) The method of claim 26, wherein the known concentration of the ceruloplasmin has at least 3 different values.

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28. (Previously added) The method of claim 11, comprising a further step of screening an antibody for neutralizing an oxidase activity of the holoceruloplasmin at the step of manufacturing the ceruloplasmin-specific monoclonal antibody.

29. (Previously added) The method of claim 11, comprising a further step of purifying the antibody after the step of manufacturing the ceruloplasmin-specific monoclonal antibody.

30. (Previously added) A method of diagnosing Wilson's disease using the screening kit of claim 20.

31. (New) The method of claim 22, wherein the absorbance standard curve according to the enzyme-linked immunosorbent assay is drawn out by applying the ceruloplasmin-specific polyclonal antibody and the ceruloplasmin-specific monoclonal antibody conjugated with horseradish peroxidase, respectively to a standard blood spot and a control reference blood spot.

32. (New) The method of claim 31, wherein the enzyme-linked immunosorbent assay for drawing out the absorbance standard curve is based on a sandwich method using the ceruloplasmin-specific polyclonal antibody and the ceruloplasmin-specific monoclonal antibody conjugated with horseradish peroxidase, respectively.

33. (New) The method of claim 23, wherein the fluorescence standard curve according to the dissociation-enhanced time-resolved fluoroimmunoassay is drawn out by applying the ceruloplasmin-specific polyclonal antibody and the ceruloplasmin-specific monoclonal antibody conjugated with europium, respectively to a standard blood spot and a control reference blood spot.

34. (New) The method of claim 33, wherein the dissociation-enhanced time-resolved fluoroimmunoassay for drawing out the fluorescence standard curve is based on a sandwich ELISA method using the ceruloplasmin specific polyclonal antibody and the ceruloplasmin-specific monoclonal antibody conjugated with europium, respectively.